ERCC2 MUTATIONS IN REPAIR-DEFICIENT HAMSTER CELLS: DIFFERENTIAL AFFINITY BETWEEN NORMAL AND MUTANT PROTEINS FOR PARTICIPATION IN THE TFIIH COMPLEX.

Saloumeh Kadkhodayan, Edmund P. Salazar, James D. Tucker, Kyoko Takayama, Marilyn Ramsey, Christine A. Weber and Larry H. Thompson, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA 94551

The ERCC2 (XPD) protein is 5' to 3' DNA helicase (1) and one component of the TFIIH complex that is required for both transcription by RNA Pol II and nucleotide excision repair. Since the *ERCC2* gene is single copy in CHO lines due to the hemizygosity of chromosome 9, this system offers advantages for understanding the phenotypes due to specific mutations. We have explored the feasibility of producing dominant negative phenotypes by overexpression of mutant alleles in wild-type CHO cells. The nucleotide sequence of the hamster *ERCC2* cDNA was determined and shown to be functional (1). The CHO mutant line, UVL-1, as well as the Chinese hamster V79-derived mutant line, V-H1, are unusual because they show high UV sensitivity combined with intermediate levels of incision and photoproduct removal. In both mutants, a single base substitution causes an a.a. substitution in ERCC2. The mutation in UVL-1 is a C223→ T transition resulting in an Arg75Trp substitution in helicase domain Ia. The equivalent a.a. position is fully conserved (Arg) in the four homologs determined so far (human, fish *Xiphophorus maculatus*, *S. cerevisiae*, and *S. pombe*). In V-H1, which has two *ERCC2* alleles, only one type of cDNA was found. The V-H1 *ERCC2* cDNA has a C137→ T transition resulting in a Thr46Ile substitution in helicase domain I, the ATP-binding domain. The equivalent a.a. position is also Thr in the four homologs. Fluorescence in situ hybridization confirmed the presence of two alleles in V-H1. Surprisingly, analysis of both cDNA and genomic DNA confirms that both alleles carry the same mutation. Site-specific mutagenesis was used to introduce the T_{137} and T_{223} cDNA changes, resulting Thr46Ile and Arg75Trp, respectively. The 46_{Ile} and 75_{Trp} cDNA plasmids each failed to confer UV resistance to the respective mutants, further demonstrating that the changes identified are the causative mutations in V-H1 and UVL-1. Furthermore, a Lys48Arg mutation in the ATPase motif (helicase domain I), was generated. The 48_{Arg} mutation in the hamster ERCC2 cDNA leads to a loss of the ability to complement the UV-sensitive phenotype of Chinese hamster group 2 mutant cells, UV5 or V-H1. Overexpression of the Lys48Arg and Thr46Ile mutant constructs in wild-type AA8 cells demonstrates a concentration dependent dominant negative effect. ERCC2 levels as high as ten fold over the wild type ERCC2 can not totally displace the wild-type protein in the complex as is evident by the survival curve data. However, over-expression of the Arg75Trp cDNA does not show a dominant negative effect. These results suggest that some mutations, such as Arg75Trp, result in a protein that can not compete efficiently for integration into the TFIIH complex, perhaps due to alterations in tertiary structure or affinity for other members of the complex. The 46_{Ile} mutation, which is predicted to result in loss of helicase activity, may allow incomplete repair that does not enhance survival. This system may be useful for studying the biochemical defect associated with mutations in XP-D and TTD individuals.
(Work was done under the auspices of the U.S. DOE by LLNL under contract No. W-7405-ENG-48 and was supported by NIH grant CA52679).

1. Sung, P., Bailly, V., Weber, C., Thompson, L. H., Prakash, L., and Prakash, S. (1993) Human xeroderma pigmentosum group D gene encodes a DNA helicase. Nature 365, 852-855.
2. Kadkhodayan, S., Salazar, E. P., Lamerdin, J. E., and Weber, C. A. (1996) Construction of a functional cDNA clone of the hamster *ERCC2* DNA repair and transcription gene. Somat. Cell Mol. Genet. in press.